DETERMINATION OF TRACE AMOUNTS OF AQUEOUS SULFIDE

BY INDIRECT CVAAS OF MERCURY

by

RICHARD A. KORTES

Submitted in Partial Fulfillment of the Requirements

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THESIS

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ABSTRACT

Determination of Trace Amounts of Aqueous Sulfide
by Indirect CVAAS of Mercury

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A method was developed by which sulfide ions could be
quantitatively determined by the stoichiometric removal of mercuric
ions from a standard solution, and the unreacted mercuric ions be
reduced to elemental mercury to be measured by cold vapor atomic
absorption. Supporting this method is the extreme insolubility of HgS
in aqueous solution.

It was found that the experimental conditions used (a pH of 3,
ascorbic acid reducing agent, a 2 L/min. carrier gas flowrate, 12 mm x
75 mm culture tube and serum sleeve cap) led to a technique capable of
determining sulfide at the 6 ppb level (in sample) with a typical
precision of ±5%.

Cationic (Cd^{2+}, Ca^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+})
and anionic (F^-, Cl^-, Br^-, I^-, CN^-, CrO_4^{2-}, CO_3^{2-}, NO_3^-, PO_4^{3-}, SO_4^{2-},
and SO_3^{2-}) interferences were evaluated to determine their
"threshold" level of interference. The cupric (Cu^{2+}) ion showed the
greatest interference effect on the system of the cations tested, even

when a 10^{-6} $\underline{M}$ stock Cu^{2+} solution was used. Zinc (Zn^{2+}) exhibited the least interfering effect on the system by the cations tested, and not even when a 10^{-2} $\underline{M}$ stock Zn^{2+} solution was used did the absorbance readings change. The most severe anionic interferences were produced by cyanide (CN^-), iodide (I^-), sulfide (SO_3^{2-}), and (to a lesser extent) bromide (Br^-). The determination of the 45.2 ppb sulfide standard was unaffected by chromate (CrO_4^{2-}), even when a stock concentration of 10^{-2} $\underline{M}$ was used. The threshold interference levels of the other ions were also determined.

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CHAPTER I

INTRODUCTION

Hydrogen sulfide (H_2S), the acidified form of the sulfide ion (S^{2-}), is a water-soluble gaseous compound which is a known poison. The sulfide ions (S^{2-} and HS^-), which are also toxic, and hydrogen sulfide are also known to cause corrosion of metals and poisoning of catalytic surfaces. For at least these three reasons, the quantitative determination of low levels of sulfide in aqueous systems is important.

Hydrogen sulfide and the sulfide ions, HS^- and S^{2-}, are known to be as toxic as cyanide.[1,2,3] In fact, both sulfide and cyanide are believed to inhibit cytochrome oxidase, an enzyme necessary for cellular respiration, and produce similar symptoms of systemic poisoning.[4] Unlike cyanide, sulfide also causes local irritation.[4] Table 1 shows the general air concentration - physiological response for acute exposure to H_2S.[1,5,6,7] Fortunately, H_2S is detectable by smell at a concentration of 0.02 parts per million (ppm), or 0.03 mg H_2S/m^3 of air.[8] However, the sense of smell cannot gauge varying concentrations of H_2S, and at 150 ppm the sense of smell is lost due to olfactory fatigue.[8] Worse, some people have a congenital inability to smell H_2S.[7] Several governmental agencies have set limits on the air concentration of H_2S for occupational exposure. The Occupational Safety and Health Administration (OSHA) has set a Permissible Exposure Limit (PEL, for 8 hour exposure) of 20 ppm, and a 10-minute exposure peak value of 50 ppm. The National Institution of Occupational Safety and Health (NIOSH) has set a Recommended Exposure

TABLE 1: H$_2$S Exposure and Physiological Effects
Exposure Time: Less Than One Hour[a]

Concentration in Air (in ppm)	Physiological Effects	
	Local (Irritation)	Systemic
50	conjunctivitis and mucus excretion in bronchioles	_____
100	nausea, breathing difficulties, photophobia and lacrimation	_____
200→250	pulmonary edema	_____
500	_____	heart palpitations, dizziness, trembling, cold sweat, headache, unconsciousness, and death (≈ 1 hour)
800→1000	_____	coma, convulsions, and death (≈ 1 min.) due to respiratory failure
> 1000		rapid death (few seconds)

[a] Compilation of information from references 6, 7, 8, and 9.

Limit (REL, also for 8 hour exposure) of 10 ppm H_2S. The American Conference of Governmental Industrial Hygienists (ACGIH) has recommended a Threshold Limit Value (TLV, for 8-hours exposure) of 10 ppm H_2S. NIOSH has also set the Immediately Dangerous to Health and Life (IDHL, the concentration at which after 30 minutes of exposure would create health effects that would hinder escaping) level for H_2S at 300 ppm.[9]

The Environmental Protection Agency (EPA) has set a concentration limit of 207 parts per billion (ppb, or µg H_2S/L of water) of undissociated H_2S for natural waters.[7]

THE RELEVANT CHEMISTRY OF SULFIDE

Sulfide in aqueous solution can exist in three forms: $H_2S(aq)$, $HS^-(aq)$, and $S^{2-}(aq)$, depending upon the pH of the solution. The equilibrium reactions of sulfide in water are:

$$H_2S(aq) \ + \ H_2O(\ell) \ \xrightarrow{K_1} \ HS^-(aq) \ + \ H_3O^+(aq) \tag{1}$$
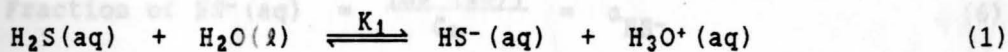
$$K_1 \ = \ \frac{[HS^-(aq)][H_3O^+(aq)]}{[H_2S(aq)][H_2O(\ell)]}$$

$$HS^-(aq) \ + \ H_2O(\ell) \ \xrightarrow{K_2} \ S^{2-}(aq) \ + \ H_3O^+(aq) \tag{2}$$
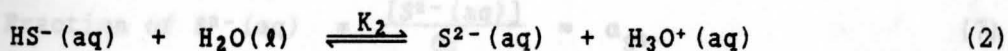
$$K_2 \ = \ \frac{[S^{2-}(aq)][H_3O^+(aq)]}{[HS^-(aq)][H_2O(\ell)]}$$

where K_1 and K_2 are equilibria constants, and the bracketed terms represent equilibrium molar concentrations. Since $[H_2O(\ell)]$ remains essentially constant (because it is the solvent and in large excess), it is traditionally combined with K_1 and K_2 to give two new constants: Ka_1 and Ka_2. Equations 3 and 4 show the equilibrium relationship between the species and Ka_1 and Ka_2, respectively. The value of the

first and second acidity constants (Ka_1 and Ka_2, respectively) are 1.0 x 10^{-7} and 1.3 x 10^{-13} at 25.0 °C.[10]

$$Ka_1 = \frac{[HS^-(aq)][H_3O^+(aq)]}{[H_2S(aq)]} \qquad (3)$$

$$Ka_2 = \frac{[S^{2-}(aq)][H_3O^+(aq)]}{[HS^-(aq)]} \qquad (4)$$

Both equilibria are occurring simultaneously in a solution of sulfide. By combining equations 3 and 4, and defining the total sulfide concentration (Cs) as the net sum of all the sulfide species in solution ($Cs = [H_2S(aq)] + [HS^-(aq)] + [S^{2-}(aq)]$), one can derive three equations (equations 5, 6, and 7) to calculate the fraction (α) of each sulfide specie as a function of pH:

$$\text{Fraction of } H_2S(aq) = \frac{[H_2S(aq)]}{Cs} = \alpha_{H_2S} \qquad (5)$$

$$= \frac{[H_3O^+(aq)]^2}{[H_3O^+(aq)]^2 + Ka_1[H_3O^+(aq)] + Ka_1Ka_2}$$

$$\text{Fraction of } HS^-(aq) = \frac{[HS^-(aq)]}{Cs} = \alpha_{HS^-} \qquad (6)$$

$$= \frac{Ka_1[H^+]}{[H_3O^+(aq)]^2 + Ka_1[H_3O^+(aq)] + Ka_1Ka_2}$$

$$\text{Fraction of } S^{2-}(aq) = \frac{[S^{2-}(aq)]}{Cs} = \alpha_{S^{2-}} \qquad (7)$$

$$= \frac{Ka_1Ka_2}{[H_3O^+(aq)]^2 + Ka_1[H_3O^+(aq)] + Ka_1Ka_2}$$

Since $pH = -\log[H_3O^+(aq)]$, then one can plot the fraction (α) of each sulfide specie versus the solution pH to produce a graph showing the major sulfide specie(s) at any pH (see Figure 1).

Since the pH affects the fraction of certain sulfide specie in a sulfide solution, and therefore the molar concentration of that specie, then other equilibria that depend upon the molar concentration

1.000

0.900

0.800

0.700

0.600

FRACTION(α)
OF
SULFIDE
SPECIE 0.500

0.400

0.300

0.200

0.100

0

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

pH

α_{H_2S} α_{HS^-} $\alpha_{S^{2-}}$
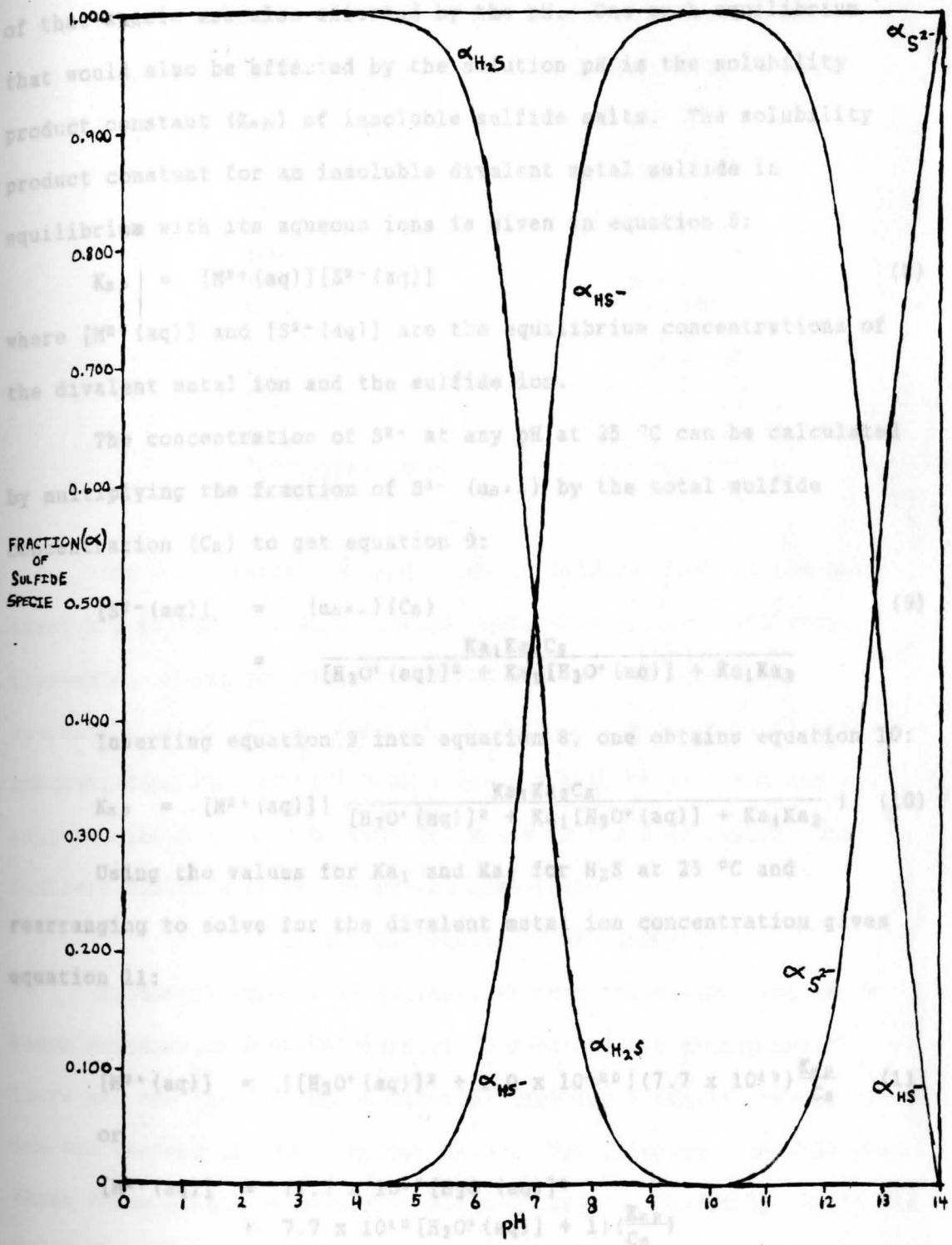
Figure 1. Fractional Variations of Aqueous Sulfide Species with
Changing Solution pH.

of that specie are also affected by the pH. One such equilibrium
that would also be affected by the solution pH is the solubility
product constant (K_{sp}) of insoluble sulfide salts. The solubility
product constant for an insoluble divalent metal sulfide in
equilibrium with its aqueous ions is given in equation 8:

$$K_{sp} = [M^{2+}(aq)][S^{2-}(aq)] \tag{8}$$

where $[M^{2+}(aq)]$ and $[S^{2-}(aq)]$ are the equilibrium concentrations of
the divalent metal ion and the sulfide ion.

The concentration of S^{2-} at any pH at 25 °C can be calculated
by multiplying the fraction of S^{2-} ($\alpha_{S^{2-}}$) by the total sulfide
concentration (C_s) to get equation 9:

$$[S^{2-}(aq)] = (\alpha_{S^{2-}})(C_s) \tag{9}$$
$$= \frac{Ka_1 Ka_2 C_s}{[H_3O^+(aq)]^2 + Ka_1[H_3O^+(aq)] + Ka_1 Ka_2}$$

Inserting equation 9 into equation 8, one obtains equation 10:

$$K_{sp} = [M^{2+}(aq)]\{ \frac{Ka_1 Ka_2 C_s}{[H_3O^+(aq)]^2 + Ka_1[H_3O^+(aq)] + Ka_1 Ka_2} \} \tag{10}$$

Using the values for Ka_1 and Ka_2 for H_2S at 25 °C and
rearranging to solve for the divalent metal ion concentration gives
equation 11:

$$[M^{2+}(aq)] = \{[H_3O^+(aq)]^2 + 1.0 \times 10^{-20}\}(7.7 \times 10^{19})\frac{K_{sp}}{C_s} \tag{11}$$

or

$$[M^{2+}(aq)] = \{7.7 \times 10^{19}[H_3O^+(aq)]^2$$
$$+ 7.7 \times 10^{12}[H_3O^+(aq)] + 1\}(\frac{K_{sp}}{C_s})$$

This equation gives the metal ion concentration in solution at
any pH for an insoluble divalent metal sulfide. Table 2 shows the K_{sp}
values for several metal sulfides at 25 °C.[11]

TABLE 2 K_{sp} Values for Some Insoluble Sulfides

Metal Sulfide	K_{sp} (at 25 °C)
CdS	8×10^{-27}
CoS,α	4×10^{-21}
CoS,β	2×10^{-25}
CuS	6×10^{-36}
FeS	6×10^{-18}
HgS	1.6×10^{-52}
MnS	3×10^{-13}
NiS,α	3×10^{-19}
NiS,β	1×10^{-24}
NiS,γ	2×10^{-26}
PbS	2.5×10^{-27}
SnS	1×10^{-25}
SnS₂	2.5×10^{-27}
ZnS(sphaelerite)	1.6×10^{-24}
ZnS(wurtzite)	5×10^{-25}

One can clearly see that mercuric sulfide, HgS, is the most insoluble sulfide. Even at low pH conditions HgS is still very insoluble. Using equation 11 and the K_{sp} for HgS, the molar concentration of Hg^{2+} in solution at a pH of 0 for a nominal sulfide concentration (C_S) of 10^{-9} $\underline{M}$ is a low 1.2×10^{-23} $\underline{M}$! This and the easy reduction of Hg^{2+} to free Hg, makes Hg^{2+} a good reagent for indirect analysis of S^{2-} by atomic absorption.

THE RELEVANT CHEMISTRY OF MERCURY

Elemental mercury is a liquid at room temperature and has a vapor pressure of 0.001201 torr at 20.0 °C and 1.0 atmosphere.[12] There are two ionic forms of mercury: mercury I (Hg_2^{2+}, or mercurous) ion and mercury II (Hg^{2+}, or mercuric). The interconversion between these three oxidation states of mercury can be done fairly easily via redox chemistry. Table 3 shows the standard reduction potentials relating the three mercury species.

TABLE 3 - Reduction Potentials of Mercury at 25 °C[13]

Reduction Reaction						E°(v) vs NHE
$Hg^{2+}(aq)$	+	$2e^-$	$\rightleftharpoons$	$Hg°(\ell)$		+0.851
$Hg_2^{2+}(aq)$	+	$2e^-$	$\rightleftharpoons$	$Hg°(\ell)$		+0.796
$2Hg^{2+}(aq)$	+	$2e^-$	$\rightleftharpoons$	$Hg_2^{2+}(eq)$		+0.905

The mercuric ion (Hg^{2+}) is the more stable and the more soluble mercury ion in aqueous solution. It forms various complex ions in solution, especially with the halides and cyanide. Table 4 shows the formation constants of the complex ions formed by Hg^{2+} with F^-, Cl^-, Br^-, I^-, and CN^-.[14]

TABLE 4 - Formation Constants for Hg^{2+} - X^- complexes at 25 °C, μ = 0.5 $\underline{M}$ for halides, 0.1 $\underline{M}$ for CN^-.[a]

X^-	HgX^+ log K_1	HgX_2 log K_2	HgX_3^- log K_3	HgX_4^{2-} log K_4
F^-	1.03	——	——	——
Cl^-	6.74	6.48	0.85	1.00
Br^-	9.05	8.28	2.41	1.26
I^-	12.87	10.95	3.67	2.37
CN^-	18.0	16.7	3.8	3.0

[a] (μ = ionic strength in moles/liter)

The fluoromercurate complex ions are virtually nonexistent, due to extensive hydrolysis of the fluoride ion. In the chloromercurate system, it appears that the $HgCl_3^-$ is favored. The bromomercurate, iodomercurate, and cyanomercurate complexes all favor the HgX_4^{2-} structure. The mercuric ion also forms neutral and cationic complexes with several other inorganic and organic ligands. Knowing the formation constants for these complex ions is important, because some of these anions are fairly common in various waters (seawater, waste water, etc.) and could cause interferences with our method by complexation of free Hg^{2+} ions prior to detection.

THE RELEVANT CHEMISTRY OF ASCORBIC ACID

L-Ascorbic acid (referred to in this work as ascorbic acid) or vitamin C, has long been known as a reducing agent. The structure of ascorbic acid shows that it has a 5-member lactone ring which contains an ene-diol structure between carbons 2 and 3. The hydroxyls at carbon 2 and 3 are responsible for both the redox properties and the acidity of ascorbic acid. Equation 12 shows ascorbic acid, the monoascorbate anion and the fully-deprotonated ascorbate anion along with the pKa's for the interconversion between the three species.[15,16]

$$pK_{a_1} = 4.0 \qquad pK_{a_2} = 11.3 \tag{12}$$

L-ascorbic acid   monoascorbate anion   fully-deprotonated ascorbate anion
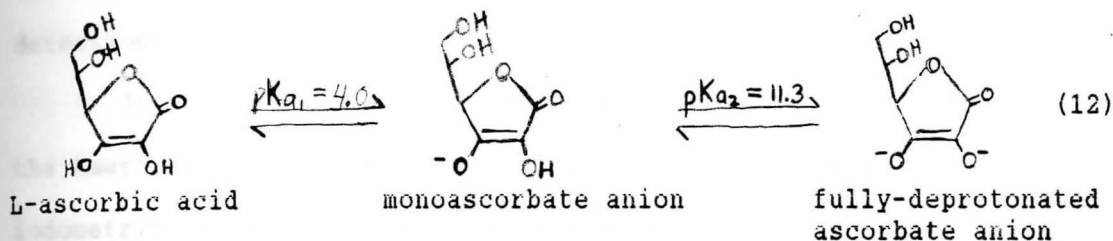
Since the hydroxyls on carbons 2 and 3 are involved with both the acidity and the redox properties, one might expect the redox properties to be affected by the pH of the solution. This has been found to be true. Standard reduction potential for the ascorbic acid/dehydroascorbic acid (the oxidized form of ascorbic acid) couple at 25 °C is +0.40 v for the reaction in equation 13:[16]

$$+ \; 2H^+ + 2e^- \rightleftharpoons \tag{13}$$

Dehydroascorbic Acid (DHA)   Ascorbic Acid (A)
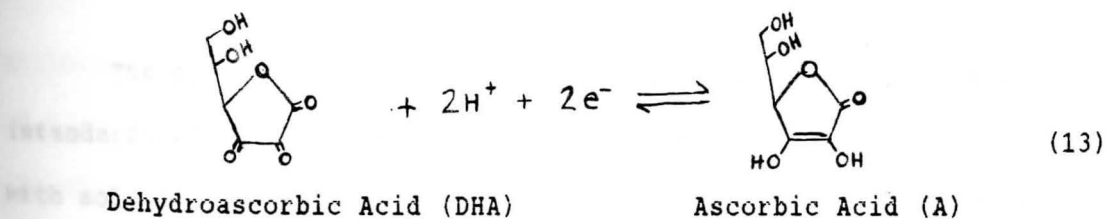
The reduction potential increases with increasing pH; therefore the potential of the reverse reaction (ascorbic acid acting as a reducing agent) becomes more negative with increasing pH.[17]

Ascorbic acid has been used in many reactions as a reducing agent, and our study is not the first to use it to reduce Hg^{2+} to elemental mercury for flameless atomic absorption.[18]

REVIEW OF THE LITERATURE

Over the years hundreds of scientific articles have been published concerning the quantitative analysis of sulfide in air and water. Considering only methods for parts-per-million (or lower) determination of sulfides, most methods can be categorized into three areas: titrimetric, electrochemical, and spectrophotometric detection.

In its Standard Methods of Analysis of Water and Wastewater, the American Public Health Association (APHA)[19] recommends the iodometric titration method for aqueous sulfide samples down to the 1 ppm (1 mg/L) concentrations. A limiting amount of the unknown sulfide sample is pipetted into a known volume of an acidified standard iodine (I_2) solution (sulfide is limiting as long as the mixture has the brown I_2 color), and a redox reaction takes place according to equation 14:

$$H_2S(aq) + I_2(aq) \rightleftharpoons S(s) + 2I^-(aq) + 2H^+(aq) \tag{14}$$
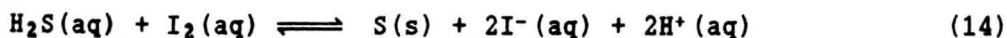
The excess I_2 is then titrated with a standard $S_2O_3^{2-}$ solution, (standardized by redox titration with the primary standard $Cr_2O_7^{2-}$), with soluble starch added as an indicator, until the deep blue color of the starch-iodine complex disappears. The concentration of S^{2-} in ppm is given by equation 15:

$$\tag{15}$$

$$ppm\ S^{2-} = \frac{(mL\ of\ I_2\ solution)(Normality\ of\ I_2)-(mL\ of\ S_2O_3^{2-})(Normality\ of\ S_2O_3^{2-})}{(mL\ of\ S^2)(1/16000)}$$

Interferences for this method include the presence of any significant amounts of reducing agents.

Another titrimetric method for analyzing low-level sulfide concentrations is potentiometric titration, utilizing a sulfide ion-selective electrode (ISE) as the end-point indicator. This procedure was employed by D. Ehman[20] to analyze parts-per-billion levels of H_2S in air. The sulfide-air standards tested were bubbled through 10 mL of ascorbate solution (0.1 $\underline{M}$ ascorbic acid in 1.0 $\underline{M}$ NaOH) to trap and preserve the H_2S as S^{2-}(aq). The solution was then titrated with a 6.00×10^{-6} $\underline{M}$ Cd^{2+} solution, and the millivolt readings from the millivolt meter (connected to the immersed ISE) were read every 30 seconds. The equivalence point, occurring at -560 millivolts, was the point of inflection on a sigmoidal plot of millivolts vs mL of Cd^{2+} solution. On the assumption of a 1:1 molar relationship between Cd^{2+} and S^{2-}, the concentration of the H_2S in the air sample could be calculated. The method was fairly linear from 50 ppb to 1000 ppb H_2S and, under the conditions employed, the detection limit was found to be 50 ppb.

Electrochemical methods of determining low levels of sulfide include potentiometry, amperometry, and coulometry, as well as other methods. One potentiometric method for determining sulfide is by use of a sulfide ion-selective electrode. Sekerka and Lechner[21] studied the characteristics (linearity, Nernstian response, limit of detection, and stability) of Ag_2S electrodes for the determination of low levels of sulfide. To stabilize the sulfide against air oxidation, which caused deviations in initial tests, an antioxidant buffer consisting of 400 g of NaOH and 500 g of ascorbic acid was

used. The detection limit for this system was about 0.5 ppb sulfide and the voltage response was Nernstian: 28 mV found, 59/2 = 28 mV theoretically for S^{2-}. No interfering substances were tested.

Han and Koch[22] employed a Dionex Model 10 ion chromatograph with amperometric detector (Dionex Model 35221) to obtain a detection limit of 0.1 ng/mL (0.1 ppb) S^{2-} and a linearity up to 1000 ppb for 100 µL of sample. In order to obtain this low detection limit and linearity range, it was necessary to modify the system and develop a workable column cleaning method. The modifications included addition of a guard column between the pump and the chromatography columns (to eliminate impurities from reagents and the pump), column cleaning with 0.5 $\underline{M}$ H_2SO_4 (high purity) to eliminate metal sulfide deposits on the column, use of a dual piston pump to decrease pulsations, and the use of two different aqueous NaH_2BO_3/Na_2CO_3/ethylenediamine eluents (one good for linear detection from 0.1 to 400 ppb and the other for the range from 1 to 1000 ppb of sulfide). No specific interferences were tested, but in early tests contaminant metal ions did interfere with the measurement.

An interesting coulometric method for the determination of S^{2-} was described by Garcès, et al.[23] This method involved the coulometric titration of sulfide by anodically-generated Hg^{2+} in a NH_4^+/NH_3 buffer at pH 9. The electrochemical reactions between the S^{2-} and the generated Hg^{2+} were measured by means of a dropping mercury electrode and the endpoint of the titration was taken from the potential vs time plot. No definite detection limit was stated, but the lowest sulfide concentration tested was 7.9 x 10^{-5} $\underline{M}$, or 2.5 ppm (made by diluting 10 µL of 0.198 $\underline{M}$ Na_2S standard with 25 mL of the

buffer). The only interferent tested was cyanide. A relative error of 1.5% was caused by a cyanide/sulfide molar ratio of approximately six (i.e., 5.0 x 10^{-3} $\underline{M}$ CN$^-$ vs 7.92 x 10^{-4} $\underline{M}$ S^{2-}).

Various spectrophotometric methods have been employed to determine part-per-million and lower levels of sulfide. Two visible spectrophotometric methods and several methods using atomic absorption spectrophotometers will be discussed.

The methylene blue method is the usual colorimetric method of choice for low level S^{2-} determination. Methylene blue is formed from the reaction between a p-aminodimethylanilinium salt and S^{2-}, catalyzed by Fe(III), as shown in equation 16:

$$H_2N-\langle\rangle-\overset{H}{\underset{\oplus}{N}}(CH_3)_2 + Cl^- + S^{2-} \xrightarrow{Fe^{3+}} \left[(CH_3)_2N-\langle\overset{N}{\underset{S}{}}\rangle-N(CH_3)_2 \right]$$

p-aminodimethylanilinium salt   Methylene Blue   (16)
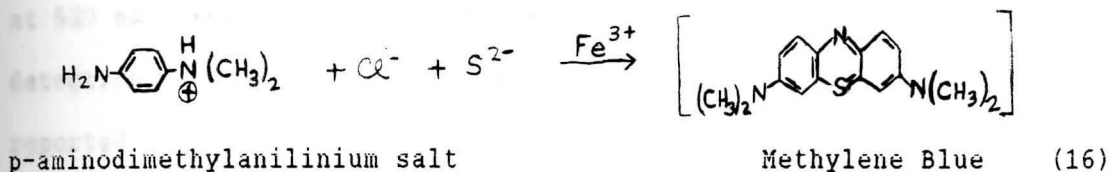
In the method proposed by Legget, et al.,[24] equal volumes of 5.4 m$\underline{M}$ p-dimethylaniline in 1.9 $\underline{M}$ HCl and 14.2 m$\underline{M}$ ferric ammonium sulfate in 0.95 $\underline{M}$ HCl are mixed by pumping each solution into a y-joint just prior to the sample injection port. The sulfide sample (65 µL) is injected through the sample port to mix with the ferric ion/amine solution. The ternary mixture is then sent to a mixing coil for color development before entering the flow-through cell in the spectrophotometer, which is set at 662 nm. The detection limit was found to be 1 ppm sulfide, and the linearity extended from 0 to 45.1 ppm S^{2-}.

Another flow-injection spectrophotometric method was described by Burguera and Townshend[25] which involved measuring light emitted

from a chemiluminescent reaction between sulfide and hypochlorite, sensitized by fluorescein. The sodium hypochlorite solution (0.1 $\underline{M}$ NaOCl and 0.01 $\underline{M}$ H_2O_2) and fluorescein solution (10^{-3} $\underline{M}$ fluorescein in a sodium carbonate buffer at pH 11.5) were pumped as separate streams to meet just before the sample injection port. A 100 µL sample of sulfide was then injected through the injection port into the mixed hypochlorite-fluorescein stream to enter a glass coil flow cell. During the 5 seconds it took for the solution to flow through the entire cell, the chemiluminescent emission peaked. A lens between the coiled cell and the spectrophotometer focused the emitted light on the entrance slit of the detecting monochromator. The readings were taken at 520 nm. For a sample volume of 1 milliliter, the limit of detection was found to be 0.4 ng/mL. The useful working range was reported to be from 1 to 1000 ng/mL of sulfide. No interfering substances were tested.

Molecular emission spectrometry has been used to quantitatively analyze sulfide in aqueous solutions. In a paper by Syty,[26] H_2S evolved from an aqueous sulfide sample after acidification is carried to a 15 cm absorption cell (with quartz windows) by a stream of nitrogen gas. The H_2S molecules were excited by a beam from a deuterium arc lamp and the emitted wavelengths were measured at 200 nm on a Perkin-Elmer Model 460 spectrophotometer with a slit width of 2 nm. The reaction vessel contained 8 mL of a 0.1 $\underline{M}$ H_2SO_4 solution into which 1.0 mL of the sulfide solution is injected via syringe through a rubber septum. The generated H_2S is carried into the absorption cell by N_2 gas at a flowrate of 1.63 L/min and the peak height measured. The solution is drained by opening a stopcock at the bottom of the

reaction vessel, while the N_2 continues to flow and clean out the absorption cell. The detection limit was stated as 1.2 µg/mL S^{2-} (or 1.2 ppm) and the absorbance vs concentration plot was linear up to 400 ppm. Several interfering anions were tested (Cl^-, Br^-, CN^-, I^-, NO_2^-, NO_3^-, CO_3^{2-}, SO_3^{2-} and CNS^-) at concentrations nearly ten times that of the S^{2-} (i.e., 3000 ppm interferent ion to 315 ppm S^{2-}). Only three ions interfered at this concentration: CN^-, SO_3^{2-}, and NO_2^-. The first two anions caused an increase in absorbance (for SO_3^{2-}, due to evolution of gases which absorb at 200 nm), and NO_2^- caused a depression in the absorbance (even though a mixture of only NO_2^- and the 0.1 $\underline{M}$ H_2SO_4 evolved some gases which also absorbed at 200 nm.

Another method employing molecular emission spectrophotometry for sulfide determination was described by Burguera and Burguera.[27] Their system consisted of a flow-injection setup (carrier stream was water) connected to a water-cooled steel cylindrical cavity that was mounted in the flame of a Varian atomic absorption spectrophotometer. The sulfide sample (2.0 µL) was injected into a stream of water (optimal flowrate of 0.14 mL min^{-1}), which then flowed into the cavity. The sulfide sample was then vaporized to form S_2 molecules, which were detected by the spectrophotometer at 384 nm (slit width 1.0 nm). The detection limit was found to be 0.02 ppm S^{2-} (for 3.0 µL sample) and the linearity of the method ranged from 0.7 to 43 ppm S^{2-}. No interferences were tested.

A very interesting method using atomic absorption coupled with a flow-injection system was reported by Petersson et al.[28] A 250 µL aliquot of known Cd^{2+} concentration (10 mg/L in 25 $\underline{mM}$ ammonium acetate buffer) was injected into a stream of the ammonium acetate buffer (pH

9.5) to mix and react with a 200 µL of sulfide sample (0.4 to 2.4 mg/L) in a second stream of buffer. The Cd^{2+} reacted with the S^{2-} to form the insoluble CdS, with the excess Cd^{2+} still in solution. The mixture then passed through a 8-quinolinol chelating ion-exchanging column to remove the excess Cd^{2+} while the suspended CdS passes through the column to go to the nebulizer. The CdS then is vaporized and atomized by the flame and the absorbance is measured at the 228.8 nm Cd line. While the absorbance was returning to the baseline level, a second pump sent another eluent (1 $\underline{M}$ HNO_3) through the ion-exchange column to release the bound Cd^{2+} and regenerate the resin. The released Cd^{2+} is then sent to the flame, and a second absorption peak is recorded. The amount of S^{2-} in the sample was equal to the amount of Cd^{2+} represented by the first of the two peaks. The reported detection limit was 0.007 mg/L, or 7 ppb. The linearity of this method appears to range from 0 to about 2.5 ppm S^{2-}. Many common ions were tested as interferents (NH_4^+, Ca^{2+}, Mg^{2+}, Cl^-, CO_3^{2-}, SCN^-, SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$ and PO_4^{3-} at 100 ppm levels; CN^- at a 2 ppm level, and I^- and Br^- at a 10 ppm level), and only PO_4^{3-} appeared to interfere at the levels tested. Coprecipitation of $Cd_3(PO_4)_2$ with the CdS was believed to be the cause.

An extremely interesting method (in view of our study) was described by Yoshida and Takahashi[29] which involved the utilization of the interference of S^{2-} on cold-vapor mercury atomic absorption determinations. Their method involved the determination of excess mercury by CVAAS at 253.7 nm (no slit width was given) in either a 2.8 cm flow-through absorption cell (25 mm diameter, quartz-windows, used for Hg at 0.2 to 35 µg amounts) or a 15.0 cm absorption cell

(25 mm diameter, quartz windows, used to determine 0.03 µg to 6 µg of Hg). The reduction vessel was 100 mL flask and a typical run had a total volume of at least 104 mL (no definite volume was given for the working mercury standard). The sulfide sample constituted 100 mL of the total volume, another 3 mL from the 50% (v/v) H_2SO_4, and 1 mL of the 10% $SnCl_2$ reducing agent. The method had a detection limit of 0.2 ppb S^{2-} and was linear from 0 to 3 ppb S^{2-}. Their calibration curve (Hg reading vs µg of S^{2-}) had a slightly more negative slope than the calculated line (just as ours did), which they attributed to adsorption of some elemental mercury by the (colloidal) HgS precipitate. They found that warming the reduction vessel to 40 °C could alleviate this problem.

THE THEORY OF COLD VAPOR ATOMIC ABSORPTION SPECTROMETRY OF MERCURY

The cold vapor atomic absorption spectrophotometry (CVAAS) method is similar to regular atomic absorption spectrophotometry (AAS) except for the atomization and vaporization procedure.

AAS involves the absorption of electromagnetic radiation by vaporized neutral atoms. The particular wavelengths of the radiation absorbed by the atoms correspond to the energies necessary to cause quantized electronic transitions from lower electron energy levels to higher (excited state) levels. The absorbed electromagnetic radiation comes from an external source (i.e., a lamp), usually having an electrode containing the element to be analyzed. When a current passes through the electrode, the electrons of the element of interest are excited to higher energy states. When the electrons return to

ground state they release energy in the form of light at frequencies characteristic of the transitions of that element. When this spectral beam of energy is absorbed by ground state analyte atoms of the same element, it causes electronic transitions of the same type. The loss of radiant power, compared to the absence of absorbing analyte atoms, is measured by a detector and results in an absorption peak.

The main analytical resonance line used for AAS analysis of mercury is the 253.7 nm line. This line represents a "forbidden" electronic transition: $^3P_1 \longrightarrow {}^1S_0$. Selection rules for electronic transitions forbid triplet to singlet transitions; however this "rule" is often violated by atoms of high atomic number. A more intense line at 184.9 nm (an allowed $^1P_1 \longrightarrow {}^1S_0$ transition) occurs for mercury;[30] however, this wavelength lies in the ultra-violet and air (O_2) tends to absorb here.

Normally, heat (flame or electric furnace) is required to atomize and vaporize the analyte ion. The flame may create absorbance problems, due to the fact that some of the combustion species may absorb at the analytical wavelength, requiring background correction. Electric furnace atomization is usually done in graphite sample containers, which may create problems due to metal carbides that may be formed between the hot graphite and the metal analyte ion.[31]

Aqueous mercuric ion can easily be chemically reduced to elemental mercury. Since elemental mercury has a fairly high vapor pressure, a certain amount of it exists in the vapor form. By passing a carrier gas through the solution containing the elemental mercury, the vaporized atomic mercury can pass through the beam of the light source while confined in a flow-through absorption cell. The mercury

can therefore be analyzed at room temperature and, since the vaporized Hg leaves the aqueous solution, there are fewer matrix interferences. It is generally known that CVAAS of mercury is a much more sensitive detection method than flame or furnace AAS.

STATEMENT OF THE PROBLEM

The need to determine low levels of sulfide in air and water is apparent on two accounts: 1) the toxicity of sulfide is well known and 2) the large number of methods developed, as can be found in the journals, for the analysis of parts-per-million (and lower) levels of sulfide.

It was decided to combine the extreme insolubility of HgS with the very sensitive method of detecting the Hg produced by the reduction of excess Hg^{2+} (aq): cold vapor atomic absorption spectrophotometry. First, a practical reduction vessel/absorption cell apparatus had to be designed. Chemically, three reducing agents were tested (ascorbic acid, sodium borohydride, and sodium dithionite), and ascorbic acid was chosen to be the reducing agent for the reduction of the excess Hg^{2+} (aq). As for most new analytical methods, the detection limit and range of linearity had to be tested. Interference threshold limits for common ions also were tested. Finally, it was felt that to test for completeness it should be determined whether the reaction between Hg^{2+} (aq) and S^{2-} (aq) to form HgS(s) was stoichiometric under the conditions used.

CHAPTER II

EXPERIMENTAL

Reagents

All reagents used were reagent grade, and the water used was ultrapure deionized water.

The stock mercury standards were prepared by dissolving reagent grade mercuric chloride (Baker A.R. #2594, 99.7% $HgCl_2$) in a small amount of water, adding 1.0 mL of 6 $\underline{M}$ HCl (GFS #660, doubly-distilled), then diluting up to 1.00 L with deionized water. A substock standard was made by pipetting (Eppendorf) 100 μL of the stock Hg^{2+} and diluting to 100.0 mL with deionized water. The substock standard was prepared fresh about once every two weeks. Working standards were prepared by diluting 1000 μL of the substock standard up to 100.0 mL with deionized water. The working standards were stored in HNO_3-rinsed plastic containers and prepared fresh every other day. The HCl was added to help stabilize the Hg^{2+} by acidification and reduction of the free Hg^{2+}(aq) ion concentration by formation of complex species ($HgCl^+$, etc.), as suggested by Koirtyohann and Khalil (although they used a higher concentration of HCl).[32] The doubly-distilled HCl was chosen because normal reagent grade HCl has been shown to contain mercury.[33]

This particular stabilization procedure was chosen because of the small effect it would have on the sulfide when mixed with the mercuric solution in the preparation of the tests. The best stabilization method for the mercury standard is in highly acidic and strongly oxidizing solutions (such as HNO_3, or HNO_3 and $K_2Cr_2O_7$).[34]

However, these are the worst conditions for S^{2-} stability. The strongly acidic conditions would make H_2S the major form of sulfide (which can be lost from the solution as a gas), and the strongly oxidizing conditions could oxidize S^{2-} to elemental S, perhaps before the reaction between Hg^{2+} and S^{2-} could occur. Also, the strongly acidic conditions would definitely have a negative effect on the reducing agents studied (ascorbic acid, sodium borohydride, and sodium dithionite).

Sulfide Standards

The stock sulfide standard was prepared by rinsing the large wet chunks of $Na_2S \cdot 9H_2O$ (Fisher Certified ACS S-425) with deionized water to remove greyish deposits of elemental sulfur (caused by air oxidation). The wet chunks were then roughly weighed and put into a one-liter plastic bottle. Two NaOH pellets (≈ 0.1 g each) were also added to the bottle and then the bottle was filled up to the 1 liter line. When all the solids dissolved, the bottle was vigorously shaken to permit thorough mixing. The stock sulfide was prepared about once a month. The stock sulfide concentrations were generally several thousand ppm. Standardization of the stock sulfide standards was done by iodometric titration (as recommended by Standard Methods of Analysis)[19] on a sample that had one-tenth the sulfide concentration of the stock (because straight stock sulfide was too concentrated). The stock solution was diluted ten-fold to obtain the first substock standard which was then several hundred ppm in sulfide. This substock was diluted again a hundred-fold to give a secondary substock of

several ppm. This standard was used to make the working sulfide standards by various dilutions. The working standards were also 0.010 $\underline{M}$ in NaOH for pH control and stabilization. The working standards were made fresh every day, or every other day.

Ascorbic Acid

A 10% ascorbic acid solution in 0.010 $\underline{M}$ NaOH was prepared by dissolving 5.0 g of analytical grade L-ascorbic acid (Baker Analyzed Reagent B581) in 5.0 mL of 0.10 $\underline{M}$ NaOH and diluting up to 50.0 mL with deionized water. This solution was stable enough for two to three days.

Other Reducing Agents

A 2% $NaBH_4$ solution was prepared by dissolving 0.50 g of Analytical Grade $NaBH_4$ (Fisher Scientific S-678) in 2.5 mL of 0.10 $\underline{M}$ NaOH and diluting up to 25 mL. The solution was stable for several days.

A 5% $Na_2S_2O_4$ solution was prepared by dissolving 5.0 g of purified (J. T. Baker #941 0609) $Na_2S_2O_4$ in 10.0 mL of 0.10 $\underline{M}$ NaOH. The solution was stable for several days.

Chemical Interference Test Solutions

These were made by dissolving the appropriate weight of salt and diluting up to the mark with deionized water. A few ($NaHCO_3$ for CO_3^{2-} test solution, NaF, Na_2HPO_4 for a PO_4^{3-} test solution) of the interference test solutions were stabilized with NaOH.

Apparatus

Atomic absorption measurements were made on an Instrument Laboratory Video 11 atomic absorption spectrophotometer. A mercury hollow cathode lamp, run at 3 milliamperes, was used as the spectral source. The slit width was set at 1.0 nm and the wavelength used was 253.7 nm. Readings were printed out on an attached strip printer. Sample tubes were mixed on a Vortex-Genie model K-550 G vortex mixer.

The Ar carrier gas was from Liquid Carbonic. The Ar flowrate was measured using a homemade soap bubble flowmeter. Figure 2 is a close-up view of the flowmeter, which consists of a 100 mL buret onto which a glass T-joint was connected by means of a short piece of Tygon tubing. On the downward arm of the T-joint was a rubber bulb half-filled with a liquid soap solution. To the side arm of the T-joint was connected a length of Tygon tubing, which was connected to the outlet of the absorption cell. Flowrates were measured, prior to any analyses, by allowing the Ar to flow through the absorption cell to the glass T-joint via the Tygon connection and then up the 100 mL buret. While the Ar was flowing, the rubber bulb was squeezed to allow the soap solution to travel upwards until it reached just past the T-joint. At this point the flowing Ar would carry the soap solution up into the buret via the buret stopcock. Here the soap bubbles form and are carried up the buret. The Ar flowrate was determined from the time (in seconds) it took for a soap bubble to travel the graduated length of the buret (i.e., 100 mL). Flowrate determinations were done in triplicate. The flowrate used in all the tests was 100 mL/3 sec, or 2 L/min. An empty tube was used in place of the reduction vessel in order to complete the Ar circuit.

rising soap bubble

Ar gas flow
from absorption cell
outlet

glass T-connection

soap solution reservoir
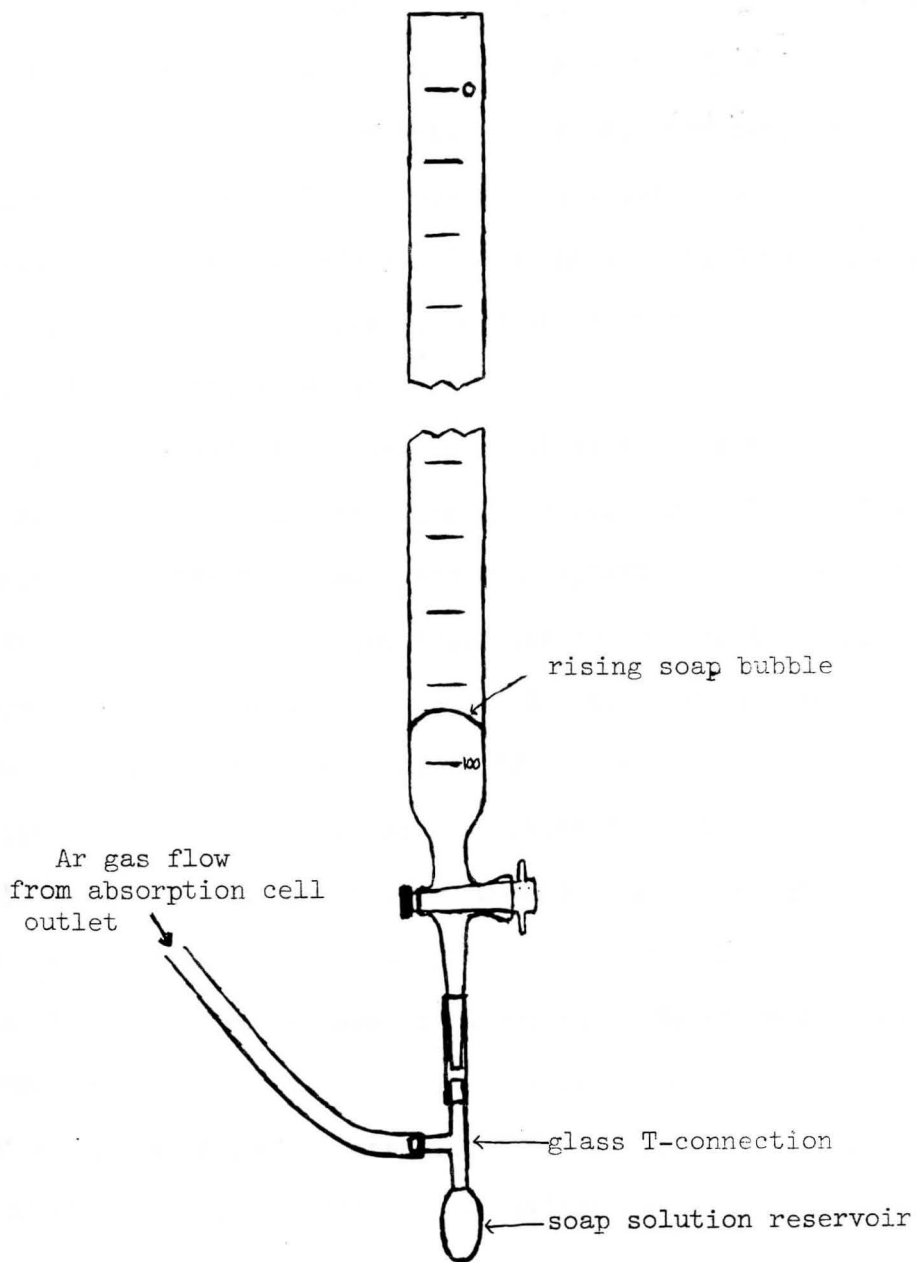
Figure 2.   Soap Bubble Flowmeter

The injector mechanism consisted of an inlet needle (21 gauge, 1.5 inches long) mounted by cyanoacrylate glue to a plexiglas plate (with hole drilled in it), and an outlet needle (16 gauge, 1.5 inch) also mounted to the plastic plate through a hole. The inlet needle was inserted only far enough for the beveled tip to be completely through the rubber septum. The injector mechanism was an adapted from of a system described by Lawrence, et al.[35] The plastic plate was mounted to a commercial pH electrode holder by a bolt and nut. The electrode holder was mounted onto a ringstand by means of a tightening screw. The reduction vessel consisted of a 12 mm x 75 mm borosilicate culture tube capped by a 13 mm rubber sleeve serum stopper (Bittner Corp.). Figure 3 shows the injector mechanism.

Three types of reduction vessels were studied. The first consisted of an interchangeable vial-injectable cap combination. The screw cap, which came with the vials, was made injectable by drilling three holes into the cap (two for the inlet and outlet needles, the third for injection of the reducing agent). A rubber septum was cut to fit inside the cap and glued into place using a cyanoacrylate glue. The second type of reduction vessel again consisted of the interchangeable vial and cap combination; however, the inlet and outlet needles were glued into the cap through drilled holes. The third hole in the cap was again used to inject the reducing agent into the vial through the septum glued into the inside of the cap. The third type of reduction vessel, described in the previous paragraph, was the one chosen to be used based on flexibility and reproducibility.

carrier gas flow to absorption cell

inlet flow of
carrier gas from tank

16 gauge needle for outlet flow

21 gauge needle for inlet
flow of carrier gas

12 mm x 75 mm culture tube reduction
vessel with 13 mm sleeve septum cap
(cutaway view to show needle insertion)
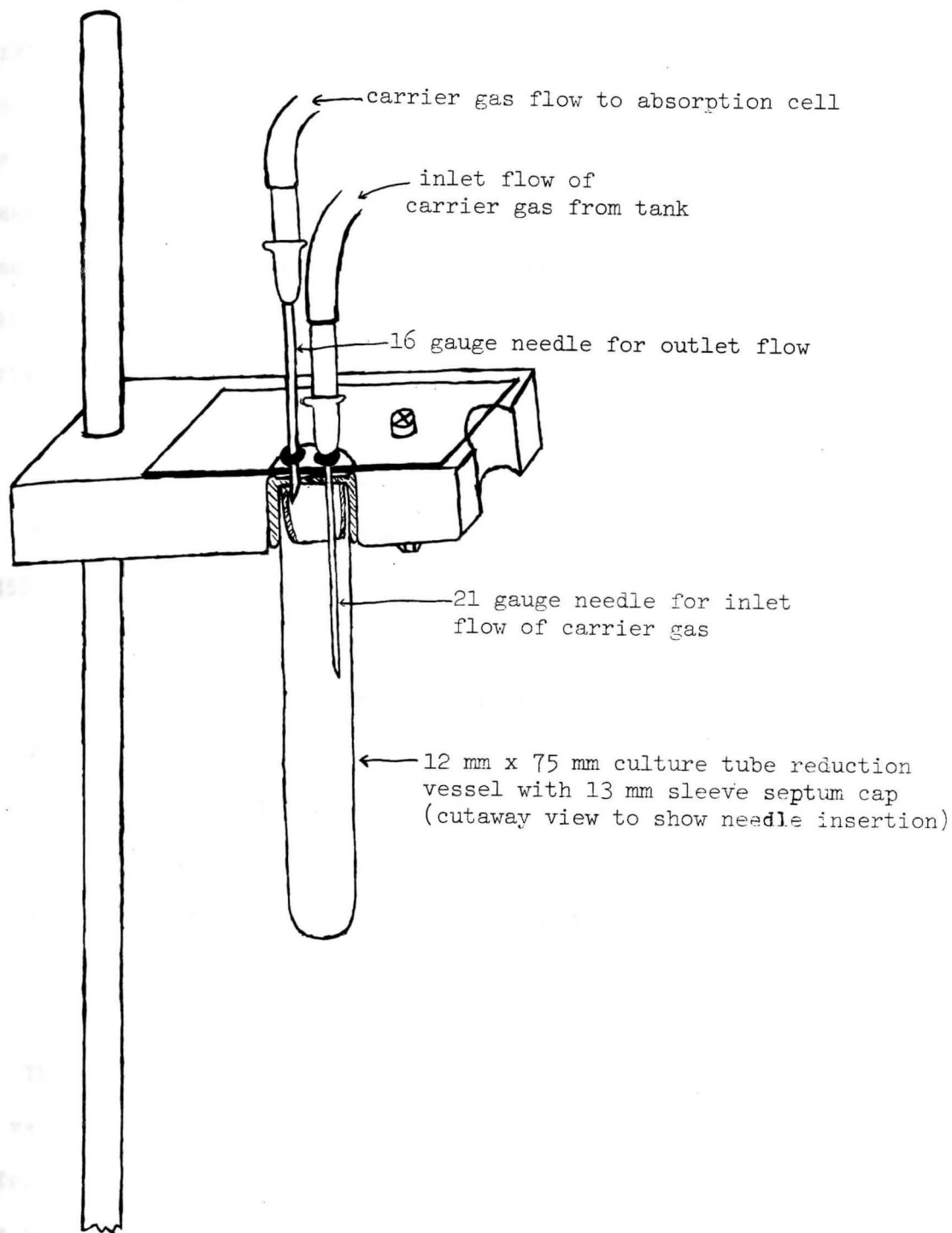
Figure 3. Injector Apparatus with an Inserted Reduction Vessel.

The absorbance cell was a commercial (Instrumentation Laboratory) quartz flow-through cell which mounts in place of the normal flame burner head. The cell itself was 15 cm in length and 2 cm in diameter. Alignment of the cell in the beam was done until a maximum transmission was achieved (indicated by the ENERGY meter). No drying tube was employed because it was deemed unnecessary due to low sample volume (450 µL total) and system flushing between runs. Two reports from the literature support this belief.[35,36]

PROCEDURE

Sample Preparation

The total volume of all sample tubes at the time of measurement was 450 µL, and are summarized below.

1. Standard ("100") mercury: 100 µL of 1.00 ppm Hg^{2+}, 250 µL H_2O, 100 µL of 10% ascorbic acid

2. Sulfide standards: 100 µL of 1.00 ppm Hg^{2+}, 50 µL H_2O, 200 µL of S^{2-} standard (variable concentration), 100 µL of 10% ascorbic acid

3. Interference tests: 100 µL of 1.00 ppm Hg^{2+}, 50 µL of interference test solution, 200 µL of S^{2-} standard (fixed concentration), and 100 µL of 10% ascorbic acid

Therefore, the total volume before the addition of the reducing agent was 350 µL. Note that the 200 µL of sulfide and the 50µL of interferent (or water) are meant to represent a 250 µL aliquot of a sample to be analyzed. All the tubes were then stoppered with the sleeve serum stoppers, and the tubes were then vortexed for 2 to 3 seconds to mix the contents. From each stoppered tube 5 mL of air was withdrawn through the septa by means of a 5 mL syringe. This was

found to increase the peak area and improve the reproducibility of the determination.

The standard "0" (blank) was analyzed and the baseline set using an empty tube with Ar flowing through. The instrument settings were as follows: the absorbance was measured using Peak Area, 20 second Integration Time, no Delay Time, in Concentration Mode, and using a Deuterium Background correction.

After the baseline was set with standard 0, the standard mercury solution (100 µL of 1.00 ppm Hg^{2+}, 250 µL of deionized water) tubes were run according to the following procedure. A syringe was used to withdraw 0.1 mL (100 µL) of the ascorbic acid reducing agent (stored in a bottle with a rubber septum cap), which was then inserted into the first standard tube and injected. The tube was immediately vortexed for 20 seconds to allow the reagents to mix. Then the tube was set aside for 10 seconds to allow the contents to equilibrate, as suggested by Lawrence, et al.[35] Toward the end of the 20 seconds, the injector needles were inserted through the septum. At the end of the 20 second period, the READ button was pushed to start the measurement and the stopcock was turned to allow the Ar to flow through the reduction vessel. The Hg atoms were carried into the absorption cell and the absorbance peaked within about 2 seconds. The reading tailed off and reached baseline again before the 20 second integration time was over. During the measurement, another 0.1 mL of ascorbic acid was drawn for the second standard tube. When the measurement was over, the first tube was replaced by a blank tube so that the system could be flushed (30 seconds) for subsequent runs. After the system was flushed, the reducing agent was injected into the second tube, and so

on. After all three or four standard "1" tubes were run, the absorbances were averaged by the Video 11 and the mean absorbance was assigned an arbitrary concentration of "100." In this way, the tubes with sulfide will read in percentages relative to the standard mercury tubes. Between sets of different samples, and after the 30 second flushing of the system, the baseline is reset to zero by use of the AUTO ZERO function on the Video 11.

CHAPTER III

DATA AND RESULTS

The important considerations in the development of a new analytical method (or modification of an existing method) are the linear range of detection, the lowest limit of detection, the sensitivity of the method, and the determination of interfering substances and the concentration at which they begin to interfere. It was also decided that the stoichiometry of the reaction between Hg^{2+} and S^{2-} and the efficacy of the three reducing agents (ascorbic acid, sodium borohydride, and sodium dithionite) should be tested.

Choice of Reducing Agent

All three reducing agents (10% ascorbic acid, 2% sodium borohydride, and 5% sodium dithionite, all in 0.01 $\underline{M}$ NaOH) were equally effective in the reduction of the mercury standard when fresh. However, sodium dithionite proved to be too pH dependent (requiring fairly high pH). Sodium borohydride worked well with both the mercury alone and the mercury/sulfide system; however, it was such a powerful reducing agent that it reduced other metal (interferent) ions to elemental form when tested in early experiments. The reduced metal interferents caused a severe reduction in the mercury absorption due to amalgamation of the elemental mercury (solution turned grey) in solution. Ascorbic acid (10% in 0.01 $\underline{M}$ NaOH) was chosen as the reducing agent because it reduced the mercuric ion to mercury just as well as did sodium borohydride and sodium dithionite, but did not seem to create the amalgamation problems in the presence of metal ion interferents. Nor did it require the high pH to function, as did

sodium dithionite. Another advantage of ascorbic acid is that it is a weak acid, and therefore will buffer the system when mixed with the higher pH sulfide solution.

Linear Range and Stoichiometry Determination

The linear range of the method was determined by using 5.6, 11.3, 22.6, 45.2, and 90.4 ppb sulfide as working standards. The average absorbance of three 1.00 ppm mercury standards (no sulfide present) was assigned a value of "100." Each of the sulfide standards was run in triplicate and the average Hg reading for each standard was reported as having a concentration relative to the "100" of the 1.00 ppm mercury standard. A plot of the average Hg absorbance vs the amount (nanograms) of sulfide in each of the standards is shown in Figure 4.

A second line (upper), representing exact 1:1 stoichiometry and 100% detection of the excess Hg^{2+} (assuming 1:1 stoichiometry between Hg^{2+} and S^{2-}) for each of the standard sulfides. Dividing the number of excess picomoles of Hg^{2+} by the total (499) picomoles of Hg^{2+} in the "100" mercury standard gives the theoretical absorbance reading for each of the sulfide standards relative to the "100" Hg standard. The experimental data and the calculated absorbance readings of the theoretical stoichiometry line can be found in Table 5.
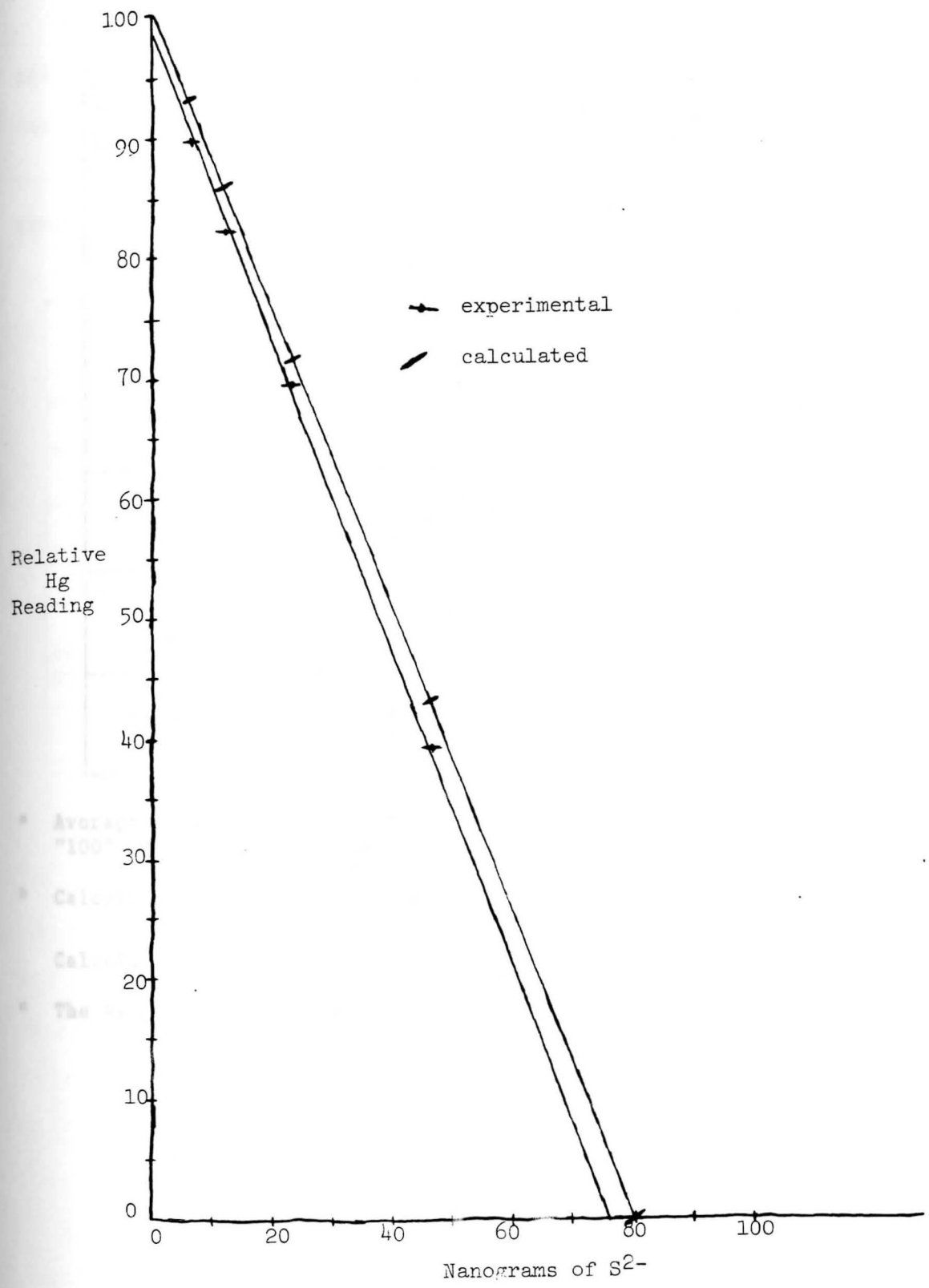
Figure 4. Calibration Plot (Relative Hg Reading vs Nanograms of Sulfide Present).

TABLE 5: Data and Calculation from Standard Curve

S^{2-} Conc. ppb	Total S^{2-} Amount (ng)	Calculated moles of S^{2-}	% of "100" Hg Standard[a] (Hg Reading) Calculated[b]	Found[c]
0	0	0	100	100
5.6	1.1	3.52×10^{-11}	92.9	89.7 ±3.0
11.3	2.3	7.05×10^{-11}	85.9	82.2 ±2.8
22.6	4.5	1.41×10^{-10}	71.7	69.3 ±5.7
45.2	9.0	2.82×10^{-10}	43.5	39.8 ±1.1
90.4	18.1	5.64×10^{-10}	-19.1	<0

[a] Average absorbance of the "100" Hg standard was 0.986 ±0.048. The "100" Hg standard contains 4.99×10^{-10} mole Hg^{2+} (499 µmole).

[b] Calculated by the following equation:

$$\text{Calculated Reading} = 100 - \frac{X \text{ moles of } S^{2-}}{4.99 \times 10^{-10} \text{ mole } Hg^{2+}}$$

[c] The average of 3 determinations.

Linear regression analysis for both plots yields the slop, x- and y- intercepts, and the correlation coefficients. This information can be found in Table 6.

TABLE 6: Linear Regression Statistics for Plotted Data in Table 5

	THEORETICAL	EXPERIMENTAL
Slope	-1.250 (ng S^{2-})$^{-1}$	-1.298 (ng S^{2-})$^{-1}$
y-intercept	100.0	98.2
x-intercept	80.0	75.7
correlation coefficient	-1.00	-0.999

Detection Limit

Defining the detection limit as the concentration of sulfide (in ppb) which causes a reduction of two standard deviations from the average "100" mercury standard, the detection limit for our method (under the conditions employed) was found to be 7 ppb S^{2-}. The data for the determination of the detection limit (run in quadruplicate) can be found in Table 7.

TABLE 7: Detection Limit Test Data

Absorbance of 1.0 ppm Hg Standards, in analyte	Relative Hg Concentration Values for 11.3 ppb S^{2-} Standard, in analyte
0.868; 0.923; 0.990; 0.899; 0.966	82.0; 87.4; 83.1; 82.4
mean: 0.929 standard deviation: 0.049 assigned value and: 100 ±5.3 relative st.dev.	mean 83.7 standard deviation: 2.5

From the results in Table 7, the two-standard deviation (2σ) detection limit should have a reading of 89.4 [i.e., 100.0-2(5.3)]. The 11.3 ppb S^{2-} standard had a mean of 83.7, which is greater than the 2σ detection limit. From the linearity data (Figure 4), a reading of 89.4 falls between the 5.6 ppb S^{2-} standard reading (89.7) and the 11.3 ppb S^{2-} standard (82.2). Interpolation between the 5.6 ppb and the 11.3 ppb readings, one obtains a detection limit of 7 ppb S^{2-}.

Sensitivity

The sensitivity of a method is the amount of response (e.g., absorbance) per unit amount of sample. For atomic abosorption spectrophotometry, the sensitivity is equal to the slope of the linear

plot of absorbance vs amount of analyte (i.e., picograms, nanomoles, etc.). Referring to Table 6, (linear regression statistics), the slope of the plot (Hg reading vs ng S^{2-}) is -1.298 Hg concentration units/ng S^{2-}. Since the "100" Hg standard had an average absorbance of 0.986 ±0.048, then multiplying the slope by 0.00986 absorbance units/Hg concentration units gives a value of -0.0128 absorbance units/ng S^{2-}. The sensitivity of the method is the absolute value of this slope, 0.0128 absorbance units/ng S^{2-}.

Interferences

Five types of interference were considered. The first type involved effects of the interfering substance on the aqueous mercury (II). Mercuric ion forms some very stable complexes with various ligands, which could prevent reduction of the Hg^{2+} by ascorbic acid. This would appear as an increased concentration of sulfide by lowering the amount of vaporized mercury in the absorption cell. Another form of interference with the aqueous mercuric ion is from ions which may act as reducing agents. These would prematurely reduce Hg^{2+} to elemental Hg, which tends to escape from the reduction vessel before the stopper can be inserted, appearing as a higher sulfide concentration.

Other types of interferences on the system were those which could interfere with the sulfide ion. Metal ion interferences could remove sulfide from the system by precipitation of insoluble metal sulfides, the degree of interference depending upon the concentration of the metal ion and the K_{sp} of the metal sulfide. Another interference on the aqueous sulfide ion could be from substances that are good oxidizing agents which could oxidize S^{2-} to elemental sulfur.

Both types of interferences would result in a lower sulfide (high mercury) interpretations.

Substances which evolve gases that absorb at 253.7 nm would cause instrumental interferences by mimicking Hg atoms. Such gases could be produced from a acid-base reaction or from a redox reaction. Such gases would be misinterpreted as a higher mercury content in the absorption cell, resulting in to low a sulfide concentration.

Chemical species which inhibit the vaporization of the elemental Hg in the aqueous solution would also constitute an interference. For the substances tested, only metal ions which could be reduced by the reducing agent would cause this problem through amalgamation by the elemental mercury. Clearly, the more powerful the reducing agent, and the greater the tendency for amalgamation, the more pronounced the effect would be.

The final type of chemical interference with which to be concerned was that which could oxidize the reducing agent before the reduction of the free Hg^{2+} to elemental mercury was complete. Anions such as nitrate and chromate, and the easily reducible metal ions would be the most likely candidates for this type of interference.

The method was checked for interferences by several metal ions (Cd^{2+}, Co^{2+}, CrO_4^{2-}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}) which are known to form insoluble sulfides or, in the case of CrO_4^{2-}, could cause interference through redox reactions. The interference of many fairly common anions (SO_3^{2-}, I^-, CN^-, CO_3^{2-}, NO_3^-, PO_4^{3-}, F^-, Cl^-, SO_4^{2-}, and Br^-) on the method were also checked. The interferences were tested against one sulfide standard which was chosen to be the 45.2 ppb S^{2-} standard. This standard was chosen because it was

located about halfway along the linear calibration curve. The goal
was to determine the threshold concentration of interfering ion which
causes a change (increase or decrease) in the average Hg reading of
the chosen sulfide standard (i.e., 45.2 ppb S^{2-}) greater than one
standard deviation but less than two standard deviations.

Tables 8, 9, and 10 contain the results from interference
testing on some metal ions, the halide and CN^- ions, and some non-
halide anions, respectively. In these tests, several 45.2 ppb S^{2-}
standard tubes (contents: 200 μL of 45.2 ppb S^{2-}, 50 μL deionized
water, 100 μL of 10% ascorbic acid) were run and the average reading
determined. Then two tubes containing the test interferent (50 μL, in
place of the deionized water) were run and the two reading were
averaged. These were then compared to the 45.2 ppb S^{2-} standard to
check agreement or disagreement.

The readings of the 45.2 ppb S^{2-} standards varied somewhat from
day to day due to aging of the three unstable reagents. Table 11
shows the concentration of the various interference and sulfide
standards in the 250 μL sample, the 350 μL volume (sample plus mecuric
standard), and the final 450 μL volume, as compared to their stock
concentrations. Also, the molar ratio of interferent to mercuric ion
(both total Hg^{2+} and the "free" Hg^{2+}, unbound by sulfide) are shown in
the table. The concentration of the 1.00 ppm mercury standard at the
various volumes is also shown.

Cationic Interferences

The cupric ion (Cu^{2+}) showed the greatest interference effect
on the system of the cations tested, even when 50 μL of 10^{-6} M stock
solution was used. In fact, no threshold level of interference was

determined for Cu^{2+} within the concentrations studied. The most likely method of interference by Cu^{2+} was through precipitation reaction with sulfide, resulting in a high Hg reading, as was observed.

Zinc (Zn^{2+}) did not exhibit an interference effect, even when stock solution of 10^{-2} $\underline{M}$ was used. In view of the low solubility product (about 10^{-24}) of ZnS, an explanation of this result could be that the low pH (about 3) prevented the K_{sp} from being exceeded.

Cadmium (Cd^{2+}) ion caused a negative (low S^{2-}) interference and its threshold effect was observed when the 10^{-6} $\underline{M}$ solution was tested. Manganous (Mn^{2+}) ion, likewise, increased the Hg reading (probably due to MnS formation) for stock Mn^{2+} solution concentrations above 10^{-5} $\underline{M}$. Nickel (Ni^{2+}) had a threshold interference concentration between 10^{-3} and 10^{-2} $\underline{M}$. Lead (Pb^{2+}) had a threshold interference concentration of about 10^{-4} $\underline{M}$. The ferric ion (Fe^{3+}) appeared to have a threshold effect somewhere between 10^{-2} and 10^{-3} $\underline{M}$ whereas Co^{2+}, Mn^{2+}, and Pb^{2+} behaved as negative interferents (possible due to formation of insoluble sulfides), both Ni^{2+} and Fe^{3+} showed positive interference effects (i.e., Hg absorbance readings were suppressed).

Anionic Interferences

The halides (Cl^-, Br^-, I^-) and CN^- all depressed the absorbance by complex formation with the free Hg^{2+}, thereby preventing the reduction of the Hg^{2+} to elemental Hg. Cyanide and iodide produced the greatest interference effects and with interference concentrations less than the lowest values tested. Chloride and bromide had threshold levels around 10^{-5} $\underline{M}$ (in the case of chloride, the stock chloride interference concentration is in addition to the small amount

of chloride already present from the mercuric standard). Fluoride had a threshold interference of 10^{-1} $\underline{M}$ stock concentration tested, and interfered by elevating the mercury absorbance over that of the mercury standard. Perhaps a small amount of HF was produced in the acidic medium which absorbs at the analytical wavelength.

In the non-halide class of anionic interferences, NO_3^- and SO_4^{2-} depressed the absorbance readings at higher concentrations, but the effect diminished when 10^{-3} $\underline{M}$ stock concentration was used for both. A possible reason for the depressed mercury absorbance is that both SO_4^{2-} and NO_3^- (which are in great excess) are known to form complexes with Hg^{2+}.[14]

The absorbance increased when CO_3^{2-} and PO_4^{3-} were tested as interferents. Carbonate still caused elevated absorbances when it was at a stock concentration of 1.0×10^{-5} $\underline{M}$. Phosphate also caused elevated readings at stock concentrations above 1.0×10^{-6} $\underline{M}$. This concentration appeared to be the threshold of interference. A speculative reason for this effect might be that these two ions somehow cause more mercury (perhaps that was adsorbed on the container wall) to be released into the vapor state.

TABLE 8: Relative Concentrations of Hg^{2+} in a Standard Hg^{2+}/S^{2-} System in the Presence of Metal Ion Interferences[a]

Conc. $\underline{M}$[b] / Ion	1.0×10^{-6}	1.0×10^{-5}	1.0×10^{-4}	1.0×10^{-3}	1.0×10^{-2}
Cd^{2+}	50.2 ±1.6 vs *49.9 ±1.5*	46.8 ±0.1 vs *48.1 ±2.9*	52.0 ±3.7 vs *46.8 ±0.8*	46.7 ±2.8 vs *39.5 ±5.1*	49.7 ±6.6 vs *48.1 ±2.9*
Co^{2+}	55.5 ±3.8 vs *55.9 ±3.8*	65.2 ±5.7 vs *49.9 ±1.5*		45.2 ±3.5 vs *31.8 ±0.4*	
CrO_4^{2-}				32.8 ±2.3 vs *31.8 ±0.4*	50.5 ±5.6 vs *49.9 ±1.5*
Cu^{2+}	54.0 ±1.1 vs *49.9 ±1.5*			80.6 ±1.1 vs *39.5 ±5.1*	
Fe^{3+}				30.4 ±0.6 vs *31.8 ±0.4*	42.7 ±2.1 vs *48.1 ±2.9*
Mn^{2+}	46.3 ±4.1 vs *49.9 ±1.5*	52.8 ±1.0 vs *55.9 ±3.8*		64.0 ±0.4 vs *39.5 ±5.1*	
Ni^{2+}				34.8 ±2.6 vs *39.5 ±5.1*	51.6 ±0.1 vs *55.9 ±3.8*
Pb^{2+}			48.3 ±4.6 vs *55.9 ±3.8*	47.1 ±1.8 vs *39.5 ±5.1*	
Zn^{2+}				38.0 ±1.3 vs *39.5 ±5.1*	52.7 ±6.1 vs *55.9 ±3.8*

[a] Tested against the 45.2 ppb S^{2-} standards, whose averages and standard deviation are the lower values shown in italics. Note that these values varied due to slight aging of the different reagents. These should be compared to the upper values in each panel, which are the results with the interferent present that were run on the same day.

[b] These were stock solutions, 50 µL of which were added to the 200 µL of 45.2 ppb sulfide standard before the mercuric standard was added. See Table 11 for conversion to final concentrations.

TABLE 9: Relative Concentrations of Hg^{2+} in a Standard Hg^{2+}/S^{2-} System in the Presence of Halide and Cyanide Interferences[a]

Conc.$\underline{M}$[b] / Ion	1.0 x 10⁻⁶	1.0 x 10⁻⁵	1.0 x 10⁻³	1.0 x 10⁻²	1.0 x 10⁻¹
F⁻				46.7 ±3.7 vs 48.1 ±2.9	66.1 ±4.1 vs[c] 61.8 ±3.2
Cl⁻	62.2 ±3.7 vs[c] 62.6 ±1.8	32.1 ±4.2 vs 41.6 ±2.2	28.2 ±0.7 vs 35.4 ±1.4		28.7 ±5.8 vs[c] 61.8 ±3.2
Br⁻	61.7 ±0.1 vs[c] 62.6 ±1.8	37.4 ±0.1 vs 41.6 ±2.2	4.2 ±1.1 vs[c] 65.4 ±2.0		‹ 0 vs[c] 61.8 ±3.2
I⁻	41.6 ±0.8 vs 48.1 ±2.9	30.1 ±2.0 vs 41.6 ±2.9	0.5 ±0.7 vs[c] 65.4 ±2.0		‹ 0 vs[c] 61.8 ±3.2
CN⁻	56.6 ±1.8 vs[c] 62.6 ±1.8		‹ 0 vs[c] 65.4 ±2.0		2.6 ±0.3 vs[c] 61.8 ±3.2

[a] Tested against the 45.2 ppb S^{2-} standard, whose averages and standard deviations are the lower values shown in italics. Note that these values vary due to aging of the differnt reagents. These should be compared to the upper values in each panel, which are the results with the interferent present, run on the same day.

[b] These were stock solutions, 50 μL of which were added to the 200 μL of 45.2 ppb sulfide standard before the mercuric standard was added. See Table 11 for conversion to final concentrations.

[c] The results came from earlier tests, before the system was well defined, in which 100 μL of 1.50 ppm Hg^{2+} was the standard used.

TABLE 11: Conversion of Stock Concentrations to the Concentrations at the Various Volumes

Stock Conc.($\underline{M}$) 1)interferent (50 µL)	in 250 µL representative sample	in 350 µL (Hg^{2+} and sample)	in 450 µL Total Volume of Analyte	Molar Ratio[a] (Substance/Hg^{2+}) Total Hg^{2+}	Free Hg^{2+}
1.0×10^{-1}	2.0×10^{-2}	1.4×10^{-2}	1.1×10^{-2}	1.0×10^4	1.8×10^4
1.0×10^{-2}	2.0×10^{-3}	1.4×10^{-3}	1.1×10^{-3}	1.0×10^3	1.8×10^3
1.0×10^{-3}	2.0×10^{-4}	1.4×10^{-4}	1.1×10^{-4}	1.0×10^2	1.8×10^2
1.0×10^{-4}	2.0×10^{-5}	1.4×10^{-5}	1.1×10^{-5}	1.0×10^1	1.8×10^1
1.0×10^{-5}	2.0×10^{-6}	1.4×10^{-6}	1.1×10^{-6}	1.0	1.8
1.0×10^{-6}	2.0×10^{-7}	1.4×10^{-7}	1.1×10^{-7}	1.0×10^{-1}	1.8×10^{-1}
2)sulfide (200 µL)					
3.5×10^{-11} (5.6 ppb S^{2-})	2.8×10^{-11}	2.0×10^{-11}	1.6×10^{-11}	3.2×10^{-2}	——
7.1×10^{-11} (11.2 ppb S^{2-})	5.6×10^{-11}	4.0×10^{-11}	3.1×10^{-11}	6.2×10^{-2}	——
1.4×10^{-10} (22.6 ppb S^{2-})	1.1×10^{-11}	8.0×10^{-11}	6.2×10^{-11}	1.3×10^{-1}	——
2.8×10^{-10} (45.2 ppb S^{2-})	2.2×10^{-10}	1.6×10^{-10}	1.2×10^{-10}	2.4×10^{-1}	——
5.6×10^{-10} (90.4 ppb S^{2-})	4.5×10^{-10}	3.2×10^{-10}	2.5×10^{-10}	5.0×10^{-1}	——
3)mercury (100 µL)					
5.00×10^{-6}		1.4×10^{-6}	1.1×10^{-6}	1.00	——

[a] S^{2-} = 2.82×10^{-10} mole for 200 µL of a 45.2 ppb S^{2-} standard. TOTAL Hg^{2+} = 4.99×10^{-10} mole Hg^{2+} for 100 µL of 1.00 ppm Hg^{2+} standard
Free Hg^{2+} = 4.99×10^{-10} mole Hg^{2+} − 2.82×10^{-10} mole Hg bound to S^{2-} = 2.17×10^{-10} mole Hg^{2+} unbound by S^{2-}.

Conclusions

The present differential method of sulfide ion analysis, based upon the loss of a stoichiometric amount of mercuric ions due to mercuric sulfide formation and subsequent reduction of the remaining mercuric ion to elemental mercury and measurement by CVAAS, was found to be able to measure sulfide concentrations to 7 ppb (with a relative standard deviation of ±5%) for interference-free samples. This method had a linear range from 0 to at least 9.0 ng S^{2-}, and a sensitivity (determined from the slope of the calibration curve) of 0.0128 absorbance units/ng S^{2-}.

The present method is novel in that it uses a mild reducing agent (ascorbic acid), which diminishes the number of metal ion interferences that might be caused by a stronger reducing agent. Also, the sample size (250 μL) is 400 times smaller than the method of indirect sulfide analysis by mercury CVAAS described by Yoshida and Takahashi.[29] Furthermore, the absolute detection limit of this method is 1.5 ng S^{2-} compared to 20 ng for the CVAAS method of the other group described above.

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